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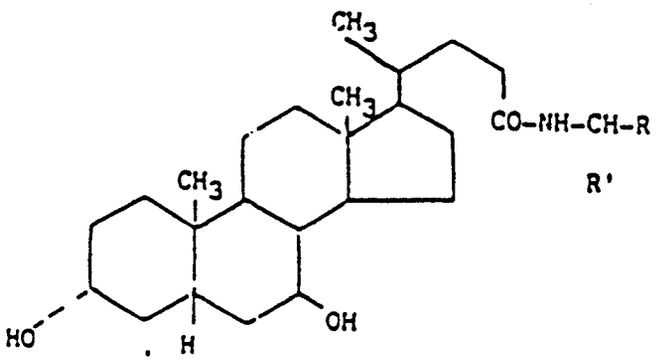
(54) Ursodeoxycholic acid derivatives and their inorganic and organic salts having therapeutic activity, and process for preparing the same.

(57) Ursodeoxycholic acid derivatives of general formula:



wherein R is a radical selected from -CH_2-SO_3H and -COOH and R' is a radical selected from -H and -(CH_2)-$_2$-CONH, -CH_2-$CONH_1$, -$(CH_2)_2$-SCH_3,-CH_2-S-CH_2 -COOH, respectively, and their salts with inorganic and organic, pharmaceutically active, bases, which are therapeutically employed mainly in the treatment of altered biligenetic functions, lithiasis or dyskinesia of biliary ducts, are prepared.
The present invention also concerns a process for preparing said derivatives.

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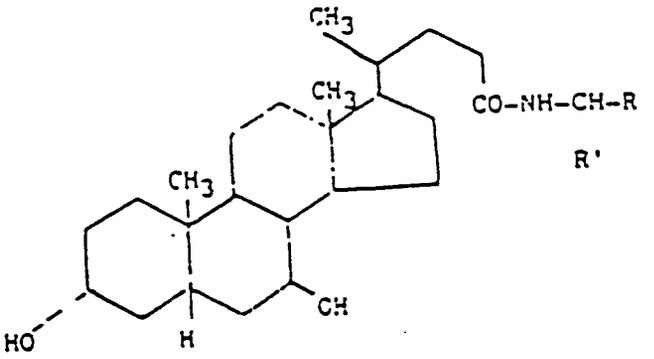
"Ursodeoxycholic acid derivatives and their inorganic and organic salts having therapeutic activity, and process for preparing the same"

The present invention concerns amides of ursodeoxycholic acid of general formula:



wherein:

$R = -CH_2-SO_3H$ $\quad R' = -H$

or:

$R = -COOH$

$R' = CH_2-CH_2-CONH_2$; $-CH_2-CONH_2$; $-CH_2-CH_2-SCH_3$;

$-CH_2-S-CH_2-COOH$

as well as their organic salts with pharmaceutically active bases.

Said derivatives are therapeutically employed mainly in the treatment of altered biligenetic functions, lithiasis or diskenesya of biliary ducts.

Tha salts of the respective derivatives with aminoacids (lysine or arginine) or with inorganic ions (Na, Ca, etc.) are also to be considered.

The presente invention also concerns the process for preparing said molecules, by way of non-limitative example, and the pharmaceutic forms containing the same.

The process for amide preparation provides for:

a) the preparation of the mixed anhydride of ursodeoxycholic acid with ethyl, phenyl, isobutyl chloroformate;

b) the reaction of said mixed anhydride with appropriate, reactive compounds, containing the $-NH_2$ group, whereby the amidic bond is formed.

Process 1° step

The anhydride of ursodeoxycholic acid is prepared while maintaining the temperature in the range between 6° and 20°C, using dioxane as solvente.

Isobutyl, phenyl or ethyl chloroformate and two moles of triethylamine are employed. The formation of anhydride takes about one hour under constant stirring.

Process II° step

The resulting mixed anhydride is reacted with the $-NH_2$ group containing reagent, at low temperature and in the presence of an organic amine. At reaction completed, appropriate treatments follow and the following derivatives are obtained:

a) when R = $-CH_2-CH_2-SO_3H$

R' = -H TAURINAMIDE

b) when R = -COOH

R' = $-(CH_2)_2-CONH_2$ GLUTAMINAMIDE

c) when R = -COOH

R' = $-CH_2-CONH_2$ ASPARAGINAMIDE

d) when R = -COOH

R' = $-CH_2-CH_2-S-CH_3$ METHIONINAMIDE

e) when R = -COOH

R' = $-CH_2-S-CH_2-COOH$ CARBOXYMETHYL-CYSTEINAMIDE.

The process according to the present invention will be now explained with reference to the following examples.

Example 1

Methioninamide of ursodeoxycholic acid

(R = -COOH; R' = $CH_2-CH_2-S-CH_3$)

a) Preparation of mixed anydride

To a suspension of 66,7 g of ursodeoxycholic acid in dioxane, 13,3 ml of ethyl chloroformate are added and the inner temperature of the system is adjusted to between 0° and 10°C.

The dioxane solution of the organic amine, preferably triethylamine, is slowly poured.

The heterogeneous, white material is then heated to room temperature.

b) Formation of amide

An aqueous solution of 27,9 g of a methionine salt with an organic amine is slowly added to the mixture resulting from a) and cooled to low temperature.

The heterogeneous, white material becomes more and more fluid, giving rise to a colourless solution when the addition is completed.

The solution temperature is allowed to increase spontaneously to 27-29°C over a course of about 5 hours, while an evolution of carbon dioxide takes place.

When the preparation of amide is completed, the product is separated by dilution with aqueous HCl and successively extracted by an organic solvent. After separation, the organic phase is treated with an aqueous solution of $NaHCO_3$ under stirring.

The two phase are separated and the aqueous solution is acidified, whereby white crystals are precipitated over a course of about 4 hours under vigorous stirring.

The resulting product, filtered under vacuum, is chromatographically pure, m.p. 107°C, with decomposition at 88-90°C.

The assay by quantitative, alkalimetric analysis is 98,51%, the impurity of free ursodeoxycholic acid being less than 0,3%. Melting point determination in a capillary tube presents a certain decomposition at 90°C, but the substance is perfectly clear a 107°C.

NMR and IR spectra prove its structural formula and are shown in the annexed Figs 1 and 2, respectively. No absorption is observed in the UV region, which happens also in the cases of compounds of the following Examples.

Example 2

Carboxymethyl-Cysteinamide of ursodeoxycholic acid

(R = -COOH; R' = $-CH_2-S-CH_2-COOH$)

The preparation follows very closely Example 1, with a sufficient amount of amine being employed to salify both the carboxy groups of carboxymethylcysteine.

The formation of amide from the mixed anhydride takes in about 2 hours and occurs at low temperature.

The amide formation is completed in about 6-8 hours.

With the procedure employed in Example 1, a product is obtained, in form of a microcrystalline, white powder, chromatographically pure, melting at 55°C;

The amount of free ursodeoxycholic acid, by quantitative alkalimetric analysis, is less then 0,5%.

NMR and IR spectra, shown in annexed Figs 3 and 4, prove its structural formula.

The melting point, determined in the capillary tube, is 55°C.

Example 3

Asparaginamide of ursodeoxychlic acid

(R = -COOH; R' = $-CH_2CONH_2$)

After the preparation of the mixed anhydride of ursodeoxycholic acid, the amide formation occurs always at low temperature by slowly adding the triethylamine salt.

In this case too the reaction is completed in about 6-8 hours.

Following the procedure of Example 1, after discharging and purification, a white crystalline product is obtained, which is chromatographically pure.

It melts at 117°C, with decomposition at 90°C.

The assay by quantitative, alkalimetric analysis is 99%, and the amount of free ursodeoxycholic acid is less than 0,5%.

NMR and IR spectra (Fig. 5 and 6) prove its structural formula.

A decomposition occurs at 90°C, but the substance is completely liquid in the capillary tube at 117°C.

Example 4

Glutaminamide of ursodeoxycholic acid

(R = -COOH; R' = $-CH_2-CH_2-CONH_2$)

The reaction of triethylamine salt of glutamine with the mixed anhydride of ursodeoxycholic acid occurs, as for the previous reactions, at low temperature, without cooling bath, over a course of about 7 hours for completion.

The mixture is then discharged as in the Example 1. White crystals are obtained, chromatographically pure and melting at 110°C, with decomposition at 85°.

The amount of free ursodeoxycholic acid is less than 0,5%, by quantitative alkalimetric analysis.

NMR and IR spectra (Figs. 7 and 8) prove its structural formula. The product decomposes at 85°C and is completely liquid ad 110°C.

Example 5

Ca salt of taurinamide of ursodeoxycholic acid

(R = $-CH_2-CH_2-SO_3H$; R' = -H)

In ths case too the reaction of the mixed anhydride of ursodeoxycholic acid is carried out by slowly adding the organic amine salt of taurine at low temperature.

After 5 hours under stirring the amide formation is completed and the reaction mixture is acidified with HCl.

The solution is then treated with $CaCO_3$.

No precipitation of the Ca salt is observed in the solution, which is then concentrated to dryness, collected in chloroform for removing the organic amine hydrochloride.

The chloroform phase is then decanted and the oily, thick, white substance is collected in acetone up to complete crystallization.

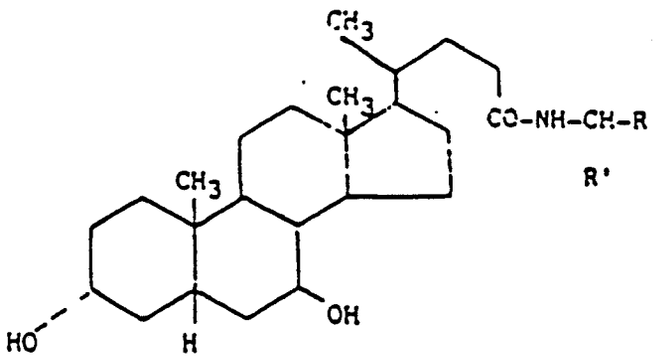
The resulting white crystals are chromatographically pure and have a melting point of 110°C;

The amount of free ursodeoxycholic acid is less than 0,5% by quantitative alkalimetric analysis.

NMR and IR spectra (Figs 9 and 10) prove its structural formula.

Claims

1. Therapeutically active derivatives of ursodeoxycholic acid of general formula:



wherein R is radical selected from $-CH_2-SO_3H$ and -COOH and R' is a radical selected from -H and $-(CH_2)_2-CONH_2$ $-CH_2-CONH_2$, $-(CH_2)_2-SCH_3$, $-CH_2-S-CH_2-COOH$, respectively, and their salts with organic and inorganic, pharmaceutically active, bases.

2. Derivatives according to claim 1, characterized in that said organic bases are basic aminoacids.

3. Derivatives according to claim 2, characterized in that said basic aminoacids are selected from lysine and arginine.

4. Derivatives according to claim 1, characterized in that said inorganic bases comprise inorganic ions.

5. Methioninamide of ursodeoxycholic acid and its salts with organic and inorganic bases according to claim 1-4.

6. Carboxymethylcisteynamide of ursodeoxycholic acid and its salts with organic and inorganic bases according to claims 1-4.

7. Asparaginamide of ursodeoxycholic acid and its salts with organic and inorganic bases according to claims 1-4.

8. Glutaminamide of ursodeoxycholic acid and its salts with organic and inorganic bases according to claims 1-4.

9. Taurinamide od ursodeoxycholic acid and its salts with organic and inorganic bases according to claims 1-4.

10. Process for preparing therapeutically active derivatives of ursodeoxycholic acid according to claim 1, characterized by the steps wherein:

a) the mixed anhydride of ursodeoxycholic acid with an alkyl chloroformate is formed, and

b) the above mixed anhydride is reacted with a reactive compound containing the $-NH_2$ group.

11. Process according to claim 10, characterized in that said alkyl chloroformate is selected from ethyl, phenyl and isobutyl chloroformate.

12. Process according to claim 10, characterized in that said step a) is carried out in the presence of an organic amine, at temperature not in excess of 20°C.

13. Process according to claim 10, characterized in that said reactive compounds are selected from organic amine salts of methionine, carboxymethylcysteine, asparagine, glutamine and taurine.

14. Process according to claim 12, characterized in that said organic amine is triethylamine.

15. Process according to claim 13, characterized in that said organic amine is triethylamine.

16. Process according to claim 10, characterized in that said step a) is carried out in dioxane as solvent.

17. Process according to claim 10, characterized in that said step b) is carried out at low temperature.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 87 11 7184

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	BE-A- 901 069 (PRODOTTI CHIMICI E ALIMENTARI SPA) * Claims; page 1 * ---	1,2,9, 13	C 07 J 41/00 A 61 K 31/575
X	CHEMICAL ABSTRACTS, vol. 51, no. 22, 25th November 1957, page 17965, abstract no. 17965e-h, Columbus, Ohio, US; T. KANAZAWA et al.: "Synthesis of ursodeoxycholic acid", & NIPPON KAGAKU ZASSHI 76, 463-5(1955) * Abstract * ---	1,2,10	
X	CHEMICAL ABSTRACTS, vol. 104, no. 7, 17th February 1986, page 539, abstract no. 51022g, Columbus, Ohio, US; & JP-A-60 161 996 (EISAI CO., LTD) 23-08-1985 * Abstract * ---	1,2,10	
X	EP-A-0 119 040 (K.K. YAKULT HONSHA) * Claims; examples * -----	1,2,10	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C 07 J 41/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	29-02-1988	HENRY J.C.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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